Rec'd PCT/PTO 23 MAY 2005

Europäisches Patentamt | European Patent Office | Office européen des brevets

14. 11. 03

REC'D 16 JAN 2004
WIPO PCT

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02079783.3

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

BEST AVAILABLE COPY

Anmeldung Nr:
Application no.: 02079783.3
Demande no:

Anmeldetag:
Date of filing: 15.11.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Tibotec Pharmaceuticals Ltd.
Unit 4, Block 4B, Blanchardstown Corporate
Park
Blanchardstown, Dublin 15
IRLANDE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Substituted indolepyridinium as anti-ifective compounds

In Anspruch genommene Prioriät(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C07D471/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

SUBSTITUTED INDOLEPYRIDINIUM AS ANTI-INFECTIVE COMPOUNDS

The present invention relates to the use of substituted indolepyridinium as anti-infective compounds, and to pharmaceutical compositions and diagnostic kits comprising them. The present invention also concerns combinations of the present substituted indolepyridinium compounds with another anti-retroviral agent. It further relates to their use in assays as reference compounds or as reagents.

The virus causing the acquired immunodeficiency syndrome (AIDS) is known by different names, including T-lymphocyte virus III (HTLV-III) or lymphadenopathy-associated virus (LAV) or AIDS-related virus (ARV) or human immunodeficiency virus (HIV). Up until now, two distinct families have been identified, i.e. HIV-1 and HIV-2. Hereinafter, HIV will be used to generically denote these viruses.

AIDS patients are currently treated with HIV protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleotide reverse transcriptase inhibitors (NtRTIs). Those compounds are often administered in drug cocktails comprising two or more compounds of the above classes of drugs. Despite the fact that these antiretrovirals are very useful, they have a common limitation, namely, the targeted enzymes in the HIV virus are able to mutate in such a way that the known drugs become less effective, or even ineffective against these mutant HIV viruses. Or, in other words, the HIV virus creates an ever-increasing resistance against the available drugs.

Resistance of retroviruses, and in particular the HIV virus, against inhibitors is a major cause of therapy failure. For instance, half of the patients receiving anti-HIV combination therapy do not respond fully to the treatment, mainly because of resistance of the virus to one or more drugs used. Moreover, it has been shown that resistant virus is carried over to newly infected individuals, resulting in severely limited therapy options for these drug-naive patients. Therefore, there is a need for new compounds for retrovirus therapy, more particularly for AIDS therapy. This need is particularly acute for compounds that are active not only on wild type HIV virus, but also on the increasingly more common resistant HIV viruses.

Known antiretrovirals, often administered in a combination therapy regimen, will eventually cause resistance as stated above. This often may force the physician to boost the plasma levels of the active drugs in order for said antiretrovirals to regain effectivity against the mutated HIV viruses. The consequence of which is a highly

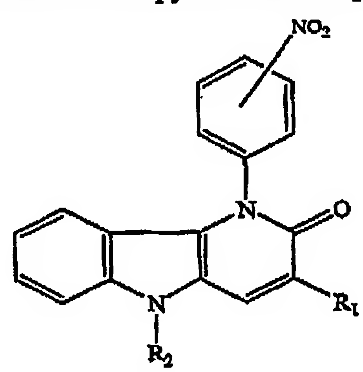
undesirable increase in pill burden. Boosting plasma levels may also lead to an increased risk of non-compliance with the prescribed therapy.

Currently used commercially available HIV reverse transcriptase inhibitors belong to three different classes, the NRTIs such as zidovudine, didanosine, zalcibatine, stavudine, abacavir and lamivudine, the NtRTIs such as tenofovir, and NNRTIs such as nevirapine, delavirdine and efavirenz. The NRTIs and NtRTIs are base analogs that target the active site of HIV reverse transcriptase (RT). Currently used NNRTI are known for rapid emergence of resistance due to mutations at amino acids that surround the NNRTI binding site (J AIDS 2001, 26, S25-S33).

Thus, there is a high medical need for anti-infective compounds that target HIV reverse transcriptase, in particular anti-retroviral compounds that are able to delay the occurrence of resistance and that combat a broad spectrum of mutants of the HIV virus.

WO 02/055520 and WO 02/059123 disclose benzoylalkylindolepyridinium compounds as antiviral compounds. Ryabova et al. disclose the synthesis of certain benzoylalkylindolepyridinium compounds (Russian Chem. Bull. 2001, 50(8), 1449-1456) (Chem. Heterocycl. Compd. (Engl.Translat.)36; 3; 2000; 301 - 306; Khim.Geterotsikl.Soedin.; RU; 3; 2000; 362 – 367).

It is now found that substituted indolepyridinium compounds of formula (I),



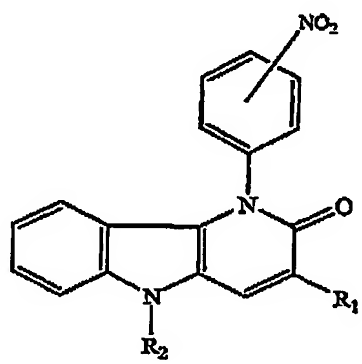
(I)

their *N*-oxides, salts, stereoisomeric forms, racemic mixtures, prodrugs, esters and metabolites,
wherein
R_1 is cyano, C_{1-4}alkylaminocarbonyl or C_{1-4}alkyloxycarbonyl;
R_2 is hydrogen or C_{1-6}alkyl; inhibit the replication of HIV virus. These compounds of formula (I) are active against wild type HIV virus and also against a variety of mutant HIV viruses including mutant HIV viruses exhibiting resistance against commercially

available reverse transcriptase (RT) inhibitors. The compounds of formula (I) are therefore useful as a medicine, and thus also useful in the manufacture of a medicament useful for preventing, treating or combating infection or disease associated with HIV infection.

A subgroup of the compounds of formula (I) is deemed novel and consists of those compounds of formula (I) provided they are different from
2,5-dihydro-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile, and
2,5-dihydro-5-methyl-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile.

Thus, the present invention also concerns the compounds of formula (I) having the formula



(I)

their *N*-oxides, salts, stereoisomeric forms, racemic mixtures, prodrugs, esters and metabolites,
wherein
R_1 is cyano, C_{1-4}alkylaminocarbonyl or C_{1-4}alkyloxycarbonyl;
R_2 is hydrogen or C_{1-6}alkyl;
provided that the compound is different from
2,5-dihydro-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile, and
2,5-dihydro-5-methyl-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile.

The term "C_{1-4}alkyl" as a group or part of a group defines straight and branched chained saturated hydrocarbon radicals having from 1 to 4 carbon atoms, such as, for example, methyl, ethyl, propyl, butyl, 2-methyl-propyl and the like.
The term "C_{1-6}alkyl" as a group or part of a group defines straight and branched chained saturated hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, the groups defined for C_{1-4}alkyl and pentyl, hexyl, 2-methylbutyl, 3-methylpentyl and the like.

The term "C_{2-6}alkyl" as a group or part of a group defines straight and branched chained saturated hydrocarbon radicals having from 2 to 6 carbon atoms such as for example, ethyl, propyl, butyl, 2-methyl-propyl, pentyl, hexyl, 2-methylbutyl, 3-methylpentyl and the like.

The term "C_{6-14}aryl" means an aromatic hydrocarbon ring having from 6 to 14 ring members such as, for example, phenyl, naphthalene, anthracene and phenanthrene.

When any variable (*e.g.* halogen or C_{1-4}alkyl) occurs more than one time in any constituent, each definition is independent.

The term "prodrug" as used throughout this text means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative is the active drug as defined in the compounds of formula (I). The reference by Goodman and Gilman (The Pharmacological Basis of Therapeutics, 8th ed, McGraw-Hill, Int. Ed. 1992, "Biotransformation of Drugs", p 13–15) describing prodrugs generally is hereby incorporated. Prodrugs of a compound of the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either by routine manipulation or in vivo, to the parent compound.

Prodrugs are characterized by excellent aqueous solubility, increased bioavailability and are readily metabolized into the active inhibitors *in vivo*.

For therapeutic use, the salts of the compounds of formula (I) are those wherein the counterion is pharmaceutically or physiologically acceptable. However, salts having a pharmaceutically unacceptable counterion may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound of formula (I). All salts, whether pharmaceutically acceptable or not are included within the ambit of the present invention.

The pharmaceutically acceptable or physiologically tolerable addition salt forms which the compounds of the present invention are able to form can conveniently be prepared using the appropriate acids, such as, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid; sulfuric; hemisulphuric, nitric; phosphoric and the like acids; or organic acids such as, for example, acetic, aspartic, dodecyl-sulphuric, heptanoic, hexanoic, nicotinic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic, maleic, fumaric, malic, tartaric, citric, methanesulfonic,

ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-amino-salicylic, pamoic and the like acids.

Conversely said acid addition salt forms can be converted by treatment with an appropriate base into the free base form.

The compounds of formula (I) containing an acidic proton may also be converted into their non-toxic metal or amine addition salt form by treatment with appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, *e.g.* the benzathine, N-methyl, -D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

Conversely said base addition salt forms can be converted by treatment with an appropriate acid into the free acid form.

The term "salts" also comprises the hydrates and the solvent addition forms that the compounds of the present invention are able to form. Examples of such forms are *e.g.* hydrates, alcoholates and the like.

The *N*-oxide forms of the present compounds are meant to comprise the compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide.

The present compounds may also exist in their tautomeric forms. Such forms, although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

The term stereochemically isomeric forms of compounds of the present invention, as used hereinbefore, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of the present invention may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms which said compound may possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of the present invention both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

Pure stereoisomeric forms of the compounds and intermediates as mentioned herein are defined as isomers substantially free of other enantiomeric or diastereomeric forms of the same basic molecular structure of said compounds or intermediates. In particular, the term 'stereoisomerically pure' concerns compounds or intermediates having a stereoisomeric excess of at least 80% (i. e. minimum 90% of one isomer and maximum 10% of the other possible isomers) up to a stereoisomeric excess of 100% (i.e. 100% of one isomer and none of the other), more in particular, compounds or intermediates having a stereoisomeric excess of 90% up to 100%, even more in particular having a stereoisomeric excess of 94% up to 100% and most in particular having a stereoisomeric excess of 97% up to 100%. The terms 'enantiomerically pure' and 'diastereomerically pure' should be understood in a similar way, but then having regard to the enantiomeric excess, respectively the diastereomeric excess of the mixture in question.

Pure stereoisomeric forms of the compounds and intermediates of this invention may be obtained by the application of art-known procedures. For instance, enantiomers may be separated from each other by the selective crystallization of their diastereomeric salts with optically active acids or bases. Examples thereof are tartaric acid, dibenzoyl-tartaric acid, ditoluoyltartaric acid and camphosulfonic acid. Alternatively, enantiomers may be separated by chromatographic techniques using chiral stationary phases. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably, if a specific stereoisomer is desired, said compound will be synthesized by stereospecific methods of preparation. These methods will advantageously employ enantiomerically pure starting materials.

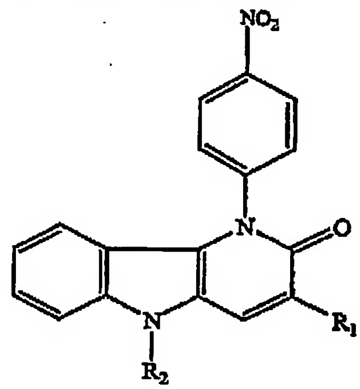
The diastereomeric racemates of formula (I) can be obtained separately by conventional methods. Appropriate physical separation methods that may advantageously be employed are, for example, selective crystallization and chromatography, e.g. column chromatography.

The present invention is also intended to include all isotopes of atoms occurring on the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.

Whenever used hereinafter, the term "compounds of formula (I)", or "the present compounds" or similar term is meant to include the compounds of general formula (I),

their *N*-oxides, salts, stereoisomeric forms, racemic mixtures, prodrugs, esters and metabolites, as well as their quaternized nitrogen analogues.

In one embodiment, the nitro group on the phenyl ring in the compound of formula (I) is in para-position vis-à-vis the nitrogen atom in the fused pyridine moiety as depicted herein below and hereinafter referred to as compounds of formula (II)



(II)

A particular group of compounds are those compounds of formula (I) wherein R_1 is cyano, methyloxycarbonyl, methylaminocarbonyl, ethyloxycarbonyl and ethylaminocarbonyl, more in particular wherein R_1 is cyano, ethyloxycarbonyl and ethylaminocarbonyl, even more in particular wherein R_1 is cyano.

Another particular group of compounds are those compounds of formula (I) wherein R_2 is hydrogen or C_{1-4}alkyl, more in particular wherein R_2 is hydrogen or methyl, even more in particular wherein R_2 is methyl.

Yet another particular group of compounds are those compounds of formula (I) wherein R_1 is cyano and R_2 is hydrogen or methyl.

A particular group of novel compounds are those compounds of formula (I) wherein R_1 is C_{1-4}alkylaminocarbonyl or C_{1-4}alkyloxycarbonyl.

Another particular group of novel compounds are those compounds of formula (I) wherein R_1 is C_{1-4}alkylaminocarbonyl or C_{1-4}alkyloxycarbonyl and R_2 is hydrogen or methyl.

Another particular group of novel compounds are those compounds of formula (I) wherein R_1 is methyloxycarbonyl, methylaminocarbonyl, ethyloxycarbonyl or ethylaminocarbonyl, and R_2 is hydrogen or methyl.

Another particular group of novel compounds are those compounds of formula (I) wherein R_2 is C_{2-6}alkyl.

Another particular group of novel compounds are those compounds of formula (I), wherein when R_1 is cyano then R_2 is different from hydrogen or methyl.

Yet another particular group of compounds are those compounds of formula (I) wherein R_2 is hydrogen or C_{1-4}alkyl, and the nitro group on the phenyl ring is in the ortho or meta position vis-à-vis the nitrogen atom in the fused pyridine moiety.

A suitable group of compounds are those compounds of formula (I) as a salt, wherein the salt is selected from trifluoroacetate, fumarate, chloroacetate, methanesulfonate, oxalate, acetate and citrate.

The compounds of the present invention inhibit the HIV reverse transcriptase and may also inhibit reverse transcriptases having similarity to HIV reverse transcriptase. Such similarity may be determined using programs known in the art including BLAST. In one embodiment, the similarity at the amino acid level is at least 25%, interestingly at least 50%, more interestingly at least 75%. In another embodiment, the similarity at the amino acid level at the binding pocket, for the compounds of the present invention, is at least 75%, in particuler at least 90% as compared to HIV reverse transcriptase.

The standard of "sensitivity" or alternatively "resistance" of a HIV reverse transcriptase enzyme to a drug is set by the commercially available HIV reverse transcriptase inhibitors. Existing commercial HIV reverse transcriptase inhibitors including efavirenz, nevirapine and delavirdine may loose effectivity over time against a population of HIV virus in a patient. The reason being that under pressure of the presence of a particular HIV reverse transcriptase inhibitor, the existing population of HIV virus, usually mainly wild type HIV reverse transcriptase enzyme, mutates into different mutants which are far less sensitive to that same HIV reverse transcriptase inhibitor. If this phenomenon occurs, one talks about resistant mutants. If those mutants are not only resistant to that one particular HIV reverse transcriptase inhibitor, but also to multiple other commercially available HIV reverse transcriptase inhibitors, one talks about multi-drug resistant HIV reverse transcriptase. One way of expressing the resistance of a mutant to a particular HIV reverse transcriptase inhibitor is making the ratio between the EC_{50} of said HIV reverse transcriptase inhibitor against mutant HIV reverse transcriptase over EC_{50} of said HIV reverse transcriptase inhibitor against

wild type HIV reverse transcriptase. Said ratio is also called fold change in resistance (FR). The EC_{50} value represents the amount of the compound required to protect 50% of the cells from the cytopathogenic effect of the virus.

Many of the mutants occurring in the clinic have a fold resistance of 100 or more against the commercially available HIV reverse transcriptase inhibitors, like didanosine, nevirapine, efavirenz, delavirdine, zalcibatine. Clinically relevant mutants of the HIV reverse transcriptase enzyme may be characterized by a mutation at codon position 100, 103 and 181. As used herein a codon position means a position of an amino acid in a protein sequence. Mutations at positions 100, 103 and 181 relate to non-nucleoside RT inhibitors (D'Aquila et al. Topics in HIV medicine, 2002, 10, 11-15). Examples of such clinical relevant mutant HIV reverse transcriptases are listed in Table 1.

<u>Table 1</u> List of mutations present in reverse transcriptase of the HIV strains used.

A	Y181C
B	K103N
C	L100I; K103N
D	L100I; K103N
E	F227C
F	Y188L
G	V106A, F227L
H	K103N, Y181C
I	K101E, K103N
J	I31L, L100I, K103N, E138G, Y181C, L214F
K	K20R, E28K, M41L, E44A, D67N, L74I, K103N, V118I, D123N, S162C, Y181C, G196K, Q207E, L210W, L214F, T215Y, K219N, P225H, D250E, P272A, R277K, I293V, P297K, K311R, R358K, T376A, E399D, T400L

An interesting group of compounds are those compounds of formula (I) having a fold resistance ranging between 0.01 and 100 against at least one mutant HIV reverse transcriptase, suitably ranging between 0.1 and 100, more suitably ranging between 0.1 and 50, and even more suitably ranging between 0.1 and 30. Of particular interest are the compounds of formula (I) showing a fold resistance against at least one mutant HIV reverse transcriptase ranging between 0.1 and 20, and even more interesting are those compounds of formula (I) showing a fold resistance against at least one mutant HIV reverse transcriptase ranging between 0.1 and 10.

An interesting group of compounds are those compounds of formula (I) having a fold resistance, determined according to the methods herein described, in the range of 0.01 to 100 against HIV species having at least one mutation in the amino acid sequence of HIV reverse transcriptase as compared to the wild type sequence (genbank accession e.g. M38432, K03455, gi 327742) at a position selected from 100, 103 and 181; in particular at least two mutations selected from the positions 100, 103 and 181. Even more interesting are those compounds within said interesting group of compounds having a fold resistance in the range of 0.1 to 100, in particular in the range 0.1 to 50, more in particular in the range 0.1 to 30. Most interesting are those compounds within said interesting group of compounds having a fold resistance in the range of 0.1 and 20, especially ranging between 0.1 and 10.

In one embodiment, the compounds of the present invention show a fold resistance in the ranges mentioned just above against at least one clinically relevant mutant HIV reverse transcriptases.

A particular group of compounds are those compounds of formula (I) having an IC_{50} of 1 μM or lower, suitably an IC_{50} of 100 nM or lower vis-à-vis the wild type virus upon *in vitro* screening according to the methods described herein.

The compounds of the present invention show antiretroviral properties, in particular against Human Immunodeficiency Virus (HIV), which is the aetiological agent of Acquired Immune Deficiency Syndrome (AIDS) in humans. The HIV virus preferentially infects CD4 receptor containing cells such as human T4 cells and destroys them or changes their normal function, particularly the coordination of the immune system. As a result, an infected patient has an ever-decreasing number of T4 cells, which moreover behave abnormally. Hence, the immunological defense system is unable to combat infections and/or neoplasms and the HIV infected subject usually dies by opportunistic infections such as pneumonia, or by cancers. Other diseases associated with HIV infection include thrombocytopaenia, Kaposi's sarcoma and infection of the central nervous system characterized by progressive demyelination, resulting in dementia and symptoms such as, progressive dysarthria, ataxia and disorientation. HIV infection further has also been associated with peripheral neuropathy, progressive generalized lymphadenopathy (PGL) and AIDS-related complex (ARC). The HIV virus also infects CD8-receptor containing cells. Other target cells for HIV virus include microglia, dendritic cells, B-cells and macrophages.

Due to their favorable pharmacological properties, particularly their activity against HIV reverse transcriptase enzymes, the compounds of the present invention or any subgroup thereof may be used as medicines against above-mentioned diseases or in the prophylaxis thereof. Said use as a medicine or method of treatment comprises the systemic administration to HIV-infected subjects of an amount effective to combat the conditions associated with HIV.

In one embodiment, the present invention concerns the use of a compound of formula (I) in the manufacture of a medicament useful for preventing, treating or combating infection or disease associated with HIV infection.

In another embodiment, the present invention concerns the use of a compound of formula (I) in the manufacture of a medicament useful for inhibiting replication of a HIV virus, in particular a HIV virus having a mutant HIV reverse transcriptase, more in particular a multi-drug resistant mutant HIV reverse transcriptase.

In yet another embodiment, the present invention relates to the use of a compound of formula (I) in the manufacture of a medicament useful for preventing, treating or combating a disease associated with HIV viral infection wherein the reverse transcriptase of the HIV virus is mutant, in particular a multi-drug resistant mutant HIV reverse transcriptase.

The compounds of formula (I) are also useful in a method for preventing, treating or combating infection or disease associated with HIV infection in a mammal, comprising administering to said mammal an effective amount of a compound of formula (I).

In another aspect, the compounds of formula (I) are useful in a method for preventing, treating or combating infection or disease associated with infection of a mammal with a mutant HIV virus, comprising administering to said mammal an effective amount of a compound of formula (I).

In another aspect, the compounds of formula (I) are useful in a method for preventing, treating or combating infection or disease associated with infection of a mammal with a multi drug-resistant HIV virus, comprising administering to said mammal an effective amount of a compound of formula (I).

In yet another aspect, the compounds of formula (I) are useful in a method for inhibiting replication of a HIV virus, in particular a HIV virus having a mutant HIV reverse transcriptase, more in particular a multi-drug resistant mutant HIV reverse

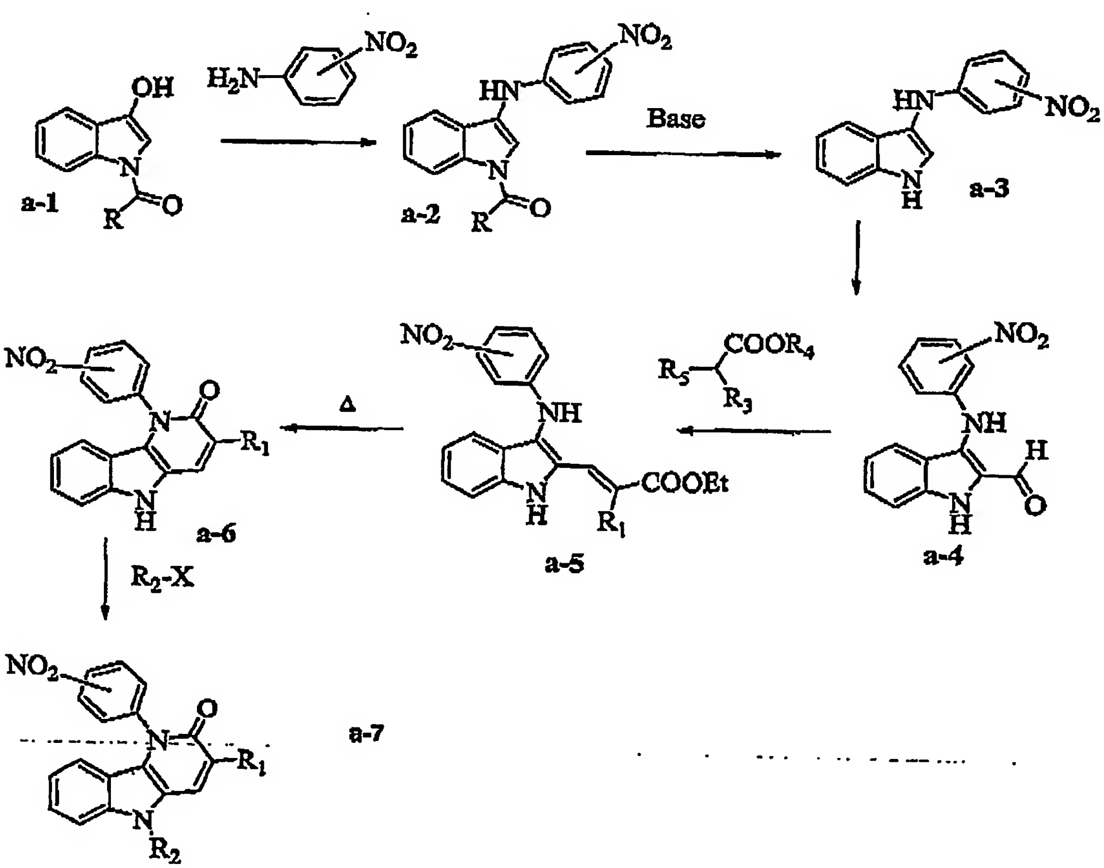
transcriptase, comprising administering to a mammal in need thereof an effective amount of a compound of formula (I).

Most interestingly, a mammal as mentioned in the present methods is a human being.

The compounds of the present invention may also find use in inhibiting *ex vivo* samples containing HIV or expected to be exposed to HIV. Hence, the present compounds may be used to inhibit HIV present in a body fluid sample that contains or is suspected to contain or be exposed to HIV.

Particular reaction procedures to make the present compounds are described below. In the preparations described below, the reaction products may be isolated from the medium and, if necessary, further purified according to methodologies generally known in the art such as, for example, extraction, crystallization, trituration and chromatography.

__Scheme A: Synthesis of Indolopyridinone compounds__



The synthesis of compounds (a-6) and (a-7) conveniently starts from 1-alkylcarbonyl-3-hydroxyindole (a-1). R in formula (a-1) represents C_{1-6}alkyl. Condensation of (a-1) with nitroaniline, at elevated temperatures yields 3-((nitrophenyl)amino)indole (a-2). In one embodiment, the nitroaniline is para-nitroaniline. Intermediate (a-2) is deacylated with a base at elevated temperature, yielding intermediate (a-3). Formylation of intermediate (a-3) results in indole aldehyde (a-4). In one embodiment, the formyl moiety is introduced using a Vilsmeier reaction. Condensation of intermediate (a-4) results in intermediate (a-5). In one embodiment, intermediate (a-4) may be reacted with a oxycarbonylmethylene reagent of formula CHR_3R_5-C(=O)-OR_4, wherein R_4 represents C_{1-6}alkyl, C_{6-14}aryl or C_{6-14}arylC_{1-6}alkyl; R_3 represents hydrogen or R_1, wherein R_1 is as defined above; R_5 represents a hydrogen, a carboxylic ester, a phosphonium salt or a phosphonate ester. In one embodiment, the reagent is of formula CH_2R_1-C(=O)-OR_4, wherein R_4 is C_{1-6}alkyl and R_1 is as defined above. Subsequent intramolecular cyclisation of intermediate (a-5) at elevated temperature, yields compound (a-6). N-alkylation using an alkyl-leaving group of formula R_2-X (wherein R_2 is as defined above and X is a leaving group) leads to compound (a-7). Examples of leaving groups include sulfonates such as tosylate, mesylate; acetates; halogens such bromide, iodide, chloride and fluoride.

The order of the above mentioned steps in said process may be different. In one approach the formylation may be performed prior to deacylation.

Oxycarbonylmethylene reagents of formula CHR_3R_5-C(=O)-OR_4 wherein R_5 represents a carboxylic ester are for instance dicarboxylic esters of formula R_4O-C(=O)-CHR_3-C(=O)-OR_4. Oxycarbonylmethylene reagents of formula CHR_3R_5-C(=O)-OR_4 wherein R_5 represents a phosphonium salt may for instance have the formula $(R_6)_3P$=CR_3-C(=O)-OR_4 wherein R_6 is C_{1-6}alkyl, C_{6-14}aryl or C_{6-14}arylC_{1-6}alkyl. Oxycarbonylmethylene reagents of formula CHR_3R_5-C(=O)-OR_4 wherein R^5 represents $(R^7O)_2P(=O)$- may for instance have the formula $(R_7O)_2P(=O)$-CHR_3-C(=O)-OR_4 wherein R_7 is C_{1-6}alkyl, C_{6-14}aryl or C_{6-14}arylC_{1-6}alkyl.

The compounds of formula (I) may also be converted to the corresponding *N*-oxide forms following art-known procedures for converting a trivalent nitrogen into its *N*-oxide form. Said *N*-oxidation reaction may generally be carried out by reacting the starting material of formula (I) with an appropriate organic or inorganic peroxide. Appropriate inorganic peroxides comprise, for example, hydrogen peroxide, alkali metal or earth alkaline metal peroxides, e.g. sodium peroxide, potassium peroxide; appropriate organic peroxides may comprise peroxy acids such as, for example,

benzenecarboperoxoic acid or halo substituted benzenecarboperoxoic acid, e.g. 3-chloro-benzenecarboperoxoic acid, peroxoalkanoic acids, e.g. peroxoacetic acid, alkylhydroperoxides, e.g. *tert*-butyl hydroperoxide. Suitable solvents are, for example, water, lower alkanols, e.g. ethanol and the like, hydrocarbons, e.g. toluene, ketones, e.g. 2-butanone, halogenated hydrocarbons, e.g. dichloromethane, and mixtures of such solvents.

A basic nitrogen occurring in the present compounds can be quaternized with any agent known to those of ordinary skill in the art including, for instance, lower alkyl halides, dialkyl sulfates, long chain halides and aralkyl halides according to art-known procedures.

The present compounds can thus be used in animals, preferably in mammals, and in particular in humans as pharmaceuticals per se, in mixtures with one another or in the form of pharmaceutical preparations.

Consequently, the present invention relates to pharmaceutical preparations that as active constituents contain an effective dose of at least one of the compounds of formula (I) in addition to customary pharmaceutically innocuous excipients and auxiliaries. The pharmaceutical preparations normally contain 0.1 to 90% by weight of a compound of formula (I). The pharmaceutical preparations can be prepared in a manner known per se to one of skill in the art. For this purpose, at least one of a compound of formula (I), together with one or more solid or liquid pharmaceutical excipients and/or auxiliaries and, if desired, in combination with other pharmaceutical active compounds, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human medicine or veterinary medicine.

Pharmaceuticals which contain a compound according to the invention can be administered orally, parenterally, e.g., intravenously, rectally, by inhalation, or topically, the preferred administration being dependent on the individual case, e.g., the particular course of the disorder to be treated. Oral administration is preferred.

The person skilled in the art is familiar on the basis of his expert knowledge with the auxiliaries that are suitable for the desired pharmaceutical formulation. Beside solvents, gel-forming agents, suppository bases, tablet auxiliaries and other active compound carriers, antioxidants, dispersants, emulsifiers, antifoams, flavor corrigents, preservatives, solubilizers, agents for achieving a depot effect, buffer substances or colorants are also useful.

Also, the combination of an antiretroviral compound and a compound of the present invention can be used as a medicine. Thus, the present invention also relates to a product containing (a) a compound of the present invention, and (b) another antiretroviral compound, as a combined preparation for simultaneous, separate or sequential use in treatment of retroviral infections such as HIV infection, in particular, in the treatment of infections with multi-drug resistant retroviruses. Thus, to prevent, combat or treat HIV infections and the disease associated with HIV infections, such as Acquired Immunodeficiency Syndrome (AIDS) or AIDS Related Complex (ARC), the compounds of this invention may be co-administered in combination with for instance, binding inhibitors, such as, for example, dextran sulfate, suramine, polyanions, soluble CD4, PRO-542, BMS-806; fusion inhibitors, such as, for example, T20, T1249, RPR 103611, YK-FH312, IC 9564, 5-helix, D-peptide ADS-J1; co-receptor binding inhibitors, such as, for example, AMD 3100, AMD-3465, AMD7049, AMD3451 (Bicyclams), TAK 779, T-22, ALX40-4C; SHC-C (SCH351125), SHC-D, PRO-140, RPR103611; RT inhibitors, such as, for example, foscarnet and prodrugs; nucleoside RTIs, such as, for example, AZT, 3TC, DDC, DDI, D4T, Abacavir, FTC, DAPD (Amdoxovir), dOTC (BCH-10652), fozivudine, DPC 817; nucleotide RTIs, such as, for example, PMEA, PMPA (tenofovir); NNRTIs, such as, for example, nevirapine, delavirdine, efavirenz, 8 and 9-Cl TIBO (tivirapine), loviride, TMC-125, dapivirine, MKC-442, UC 781, UC 782, Capravirine, QM96521, GW420867X, DPC 961, DPC963, DPC082, DPC083, TMC-125, calanolide A, SJ-3366, TSAO, 4"-deaminated TSAO, MV150, MV026048, PNU-142721; RNAse H inhibitors, such as, for example, SP1093V, PD126338; TAT inhibitors, such as, for example, RO-5-3335, K12, K37; integrase inhibitors, such as, for example, L 708906, L 731988, S-1360; protease inhibitors, such as, for example, amprenavir and prodrug GW908, ritonavir, nelfinavir, saquinavir, indinavir, lopinavir, palinavir, BMS 186316, atazanavir, DPC 681, DPC 684, tipranavir, AG1776, mozenavir, DMP-323, GS3333, KNI-413, KNI-272, L754394, L756425, LG-71350, PD161374, PD173606, PD177298, PD178390, PD178392, PNU 140135, TMC-114, maslinic acid, U-140690; glycosylation inhibitors, such as, for example, castanospermine, deoxynojirimycine; entry inhibitors CGP64222.

The combination may provide a synergistic effect, whereby viral infectivity and its associated symptoms may be prevented, substantially reduced, or eliminated completely.

The compounds of the present invention may also be administered in combination with immunomodulators (e.g., bropirimine, anti-human alpha interferon antibody, IL-2, methionine enkephalin, interferon alpha, and naltrexone) with antibiotics (e.g.,

pentamidine isothiorate) cytokines (e.g. Th2), modulators of cytokines, chemokines or modulators of chemokines, chemokine receptors (e.g. CCR5, CXCR4), modulators chemokine receptors, or hormones (e.g. growth hormone) to ameliorate, combat, or eliminate HIV infection and its symptoms. Such combination therapy in different formulations, may be administered simultaneously, sequentially or independently of each other. Alternatively, such combination may be administered as a single formulation, whereby the active ingredients are released from the formulation simultaneously or separately.

The compounds of the present invention may also be administered in combination with modulators of the metabolization following application of the drug to an individual. These modulators include compounds that interfere with the metabolization at cytochromes, such as cytochrome P450. It is known that several isoenzymes exist of cytochrome P450, one of which is cytochrome P450 3A4. Ritonavir is an example of a modulator of metabolization via cytochrome P450. Such combination therapy in different formulations, may be administered simultaneously, sequentially or independently of each other. Alternatively, such combination may be administered as a single formulation, whereby the active ingredients are released from the formulation simultaneously or separately. Such modulator may be administered at the same or different ratio as the compound of the present invention. Preferably, the weight ratio of such modulator vis-à-vis the compound of the present invention (modulator:compound of the present invention) is 1:1 or lower, more preferable the ratio is 1:3 or lower, suitably the ratio is 1:10 or lower, more suitably the ratio is 1:30 or lower.

For an oral administration form, compounds of the present invention are mixed with suitable additives, such as excipients, stabilizers or inert diluents, and brought by means of the customary methods into the suitable administration forms, such as tablets, coated tablets, hard capsules, aqueous, alcoholic, or oily solutions. Examples of suitable inert carriers are gum arabic, magnesia, magnesium carbonate, potassium phosphate, lactose, glucose, or starch, in particular, corn starch. In this case the preparation can be carried out both as dry and as moist granules. Suitable oily excipients or solvents are vegetable or animal oils, such as sunflower oil or cod liver oil. Suitable solvents for aqueous or alcoholic solutions are water, ethanol, sugar solutions, or mixtures thereof. Polyethylene glycols and polypropylene glycols are also useful as further auxiliaries for other administration forms.

For subcutaneous or intravenous administration, the active compounds, if desired with the substances customary therefor such as solubilizers, emulsifiers or further

auxiliaries, are brought into solution, suspension, or emulsion. The compounds of formula (I) can also be lyophilized and the lyophilizates obtained used, for example, for the production of injection or infusion preparations. Suitable solvents are, for example, water, physiological saline solution or alcohols, e.g. ethanol, propanol, glycerol, in addition also sugar solutions such as glucose or mannitol solutions, or alternatively mixtures of the various solvents mentioned.

Suitable pharmaceutical formulations for administration in the form of aerosols or sprays are, for example, solutions, suspensions or emulsions of the compounds of formula (I) or their physiologically tolerable salts in a pharmaceutically acceptable solvent, such as ethanol or water, or a mixture of such solvents. If required, the formulation can also additionally contain other pharmaceutical auxiliaries such as surfactants, emulsifiers and stabilizers as well as a propellant. Such a preparation customarily contains the active compound in a concentration from approximately 0.1 to 50%, in particular from approximately 0.3 to 3% by weight.

In order to enhance the solubility and/or the stability of the compounds of formula (I) in pharmaceutical compositions, it can be advantageous to employ α-, β- or γ-cyclodextrins or their derivatives. Also co-solvents such as alcohols may improve the solubility and/or the stability of the compounds of formula (I) in pharmaceutical compositions. In the preparation of aqueous compositions, addition salts of the subject compounds are obviously more suitable due to their increased water solubility.

Appropriate cyclodextrins are α-, β- or γ-cyclodextrins (CDs) or ethers and mixed ethers thereof wherein one or more of the hydroxy groups of the anhydroglucose units of the cyclodextrin are substituted with C_{1-6}alkyl, particularly methyl, ethyl or isopropyl, e.g. randomly methylated β-CD; hydroxyC_{1-6}alkyl, particularly hydroxyethyl, hydroxypropyl or hydroxybutyl; carboxyC_{1-6}alkyl, particularly carboxymethyl or carboxyethyl; C_{1-6}alkyl–carbonyl, particularly acetyl; C_{1-6}alkyloxycarbonylC_{1-6}alkyl or carboxyC_{1-6}alkyloxyC_{1-6}alkyl, particularly carboxymethoxypropyl or carboxyethoxypropyl; C_{1-6}alkylcarbonyloxyC_{1-6}alkyl, particularly 2-acetyloxypropyl. Especially noteworthy as complexants and/or solubilizers are β-CD, randomly methylated β-CD, 2,6-dimethyl-β-CD, 2-hydroxyethyl-β-CD, 2-hydroxyethyl-γ-CD, 2-hydroxypropyl-γ-CD and (2-carboxymethoxy)propyl-β-CD, and in particular 2-hydroxypropyl-β-CD (2-HP-β-CD).

The term mixed ether denotes cyclodextrin derivatives wherein at least two cyclodextrin hydroxy groups are etherified with different groups such as, for example, hydroxy-propyl and hydroxyethyl.

An interesting way of formulating the present compounds in combination with a cyclodextrin or a derivative thereof has been described in EP-A-721,331. Although the formulations described therein are with antifungal active ingredients, they are equally interesting for formulating the compounds of the present invention. The formulations described therein are particularly suitable for oral administration and comprise an antifungal as active ingredient, a sufficient amount of a cyclodextrin or a derivative thereof as a solubilizer, an aqueous acidic medium as bulk liquid carrier and an alcoholic co-solvent that greatly simplifies the preparation of the composition. Said formulations may also be rendered more palatable by adding pharmaceutically acceptable sweeteners and/or flavours.

Other convenient ways to enhance the solubility of the compounds of the present invention in pharmaceutical compositions are described in WO 94/05263, WO 98/42318, EP-A-499,299 and WO 97/44014, all incorporated herein by reference.

More in particular, the present compounds may be formulated in a pharmaceutical composition comprising a therapeutically effective amount of particles consisting of a solid dispersion comprising (a) a compound of formula (I), and (b) one or more pharmaceutically acceptable water-soluble polymers.

The term "a solid dispersion" defines a system in a solid state (as opposed to a liquid or gaseous state) comprising at least two components, wherein one component is dispersed more or less evenly throughout the other component or components. When said dispersion of the components is such that the system is chemically and physically uniform or homogenous throughout or consists of one phase as defined in thermo-dynamics, such a solid dispersion is referred to as "a solid solution". Solid solutions are preferred physical systems because the components therein are usually readily bioavailable to the organisms to which they are administered.

The term "a solid dispersion" also comprises dispersions which are less homogenous throughout than solid solutions. Such dispersions are not chemically and physically uniform throughout or comprise more than one phase.

The water-soluble polymer in the particles is conveniently a polymer that has an apparent viscosity of 1 to 100 mPa.s when dissolved in a 2 % aqueous solution at 20°C solution.

Preferred water-soluble polymers are hydroxypropyl methylcelluloses or HPMC. HPMC having a methoxy degree of substitution from about 0.8 to about 2.5 and a hydroxypropyl molar substitution from about 0.05 to about 3.0 are generally water soluble. Methoxy degree of substitution refers to the average number of methyl ether groups present per anhydroglucose unit of the cellulose molecule. Hydroxy-propyl molar substitution refers to the average number of moles of propylene oxide which have reacted with each anhydroglucose unit of the cellulose molecule.

The particles as defined hereinabove can be prepared by first preparing a solid dispersion of the components, and then optionally grinding or milling that dispersion. Various techniques exist for preparing solid dispersions including melt-extrusion, spray-drying and solution-evaporation, melt-extrusion being preferred.

It may further be convenient to formulate the present compounds in the form of nanoparticles which have a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than 1000 nm. Useful surface modifiers are believed to include those that physically adhere to the surface of the antiretroviral agent but do not chemically bond to the antiretroviral agent.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants.

Yet another interesting way of formulating the present compounds involves a pharmaceutical composition whereby the present compounds are incorporated in hydrophilic polymers and applying this mixture as a coat film over many small beads, thus yielding a composition with good bioavailability which can conveniently be manufactured and which is suitable for preparing pharmaceutical dosage forms for oral administration.

Said beads comprise (a) a central, rounded or spherical core, (b) a coating film of a hydrophilic polymer and an antiretroviral agent and (c) a seal-coating polymer layer.

Materials suitable for use as cores in the beads are manifold, provided that said materials are pharmaceutically acceptable and have appropriate dimensions and firmness. Examples of such materials are polymers, inorganic substances, organic substances, and saccharides and derivatives thereof.

The route of administration may depend on the condition of the subject, co-medication and the like.

Another aspect of the present invention concerns a kit or container comprising a compound of formula (I) in an amount effective for use as a standard or reagent in a test or assay for determining the ability of a potential pharmaceutical to inhibit HIV reverse transcriptase, HIV growth, or both. This aspect of the invention may find its use in pharmaceutical research programs.

The compounds of the present invention can be used in phenotypic resistance monitoring assays, such as known recombinant assays, in the clinical management of resistance developing diseases such as HIV. A particularly useful resistance monitoring system is a recombinant assay known as the Antivirogram®. The Antivirogram® is a highly automated, high throughput, second generation, recombinant assay that can measure susceptibility, especially viral susceptibility, to the compounds of the present invention. (Hertogs K *et al. Antimicrob Agents Chemother*, 1998; 42(2):269-276, incorporated by reference).

Interestingly, the compounds of the present invention may comprise chemically reactive moieties capable of forming covalent bonds to localized sites such that said compound have increased tissue retention and half-lives. The term "chemically reactive group" as used herein refers to chemical groups capable of forming a covalent bond. Reactive groups will generally be stable in an aqueous environment and will usually be carboxy, phosphoryl, or convenient acyl group, either as an ester or a mixed anhydride, or an imidate, or a maleimidate thereby capable of forming a covalent bond with functionalities such as an amino group, a hydroxy or a thiol at the target site on for example blood components such as albumine. The compounds of the present invention may be linked to maleimide or derivatives thereof to form conjugates.

The dose of the present compounds or of the physiologically tolerable salt(s) thereof to be administered depends on the individual case and, as customary, is to be adapted to the conditions of the individual case for an optimum effect. Thus it depends, of course, on the frequency of administration and on the potency and duration of action of the compounds employed in each case for therapy or prophylaxis, but also on the nature and severity of the infection and symptoms, and on the sex, age, weight co-medication and individual responsiveness of the human or animal to be treated and on whether the therapy is acute or prophylactic. Customarily, the daily dose of a compound of formula (I) in the case of administration to a patient approximately 75 kg in weight is 1 mg to

1g, preferably 3 mg to 0.5 g. The dose can be administered in the form of an individual dose, or divided into several, e.g. two, three, or four, individual doses.

Legends to the figures

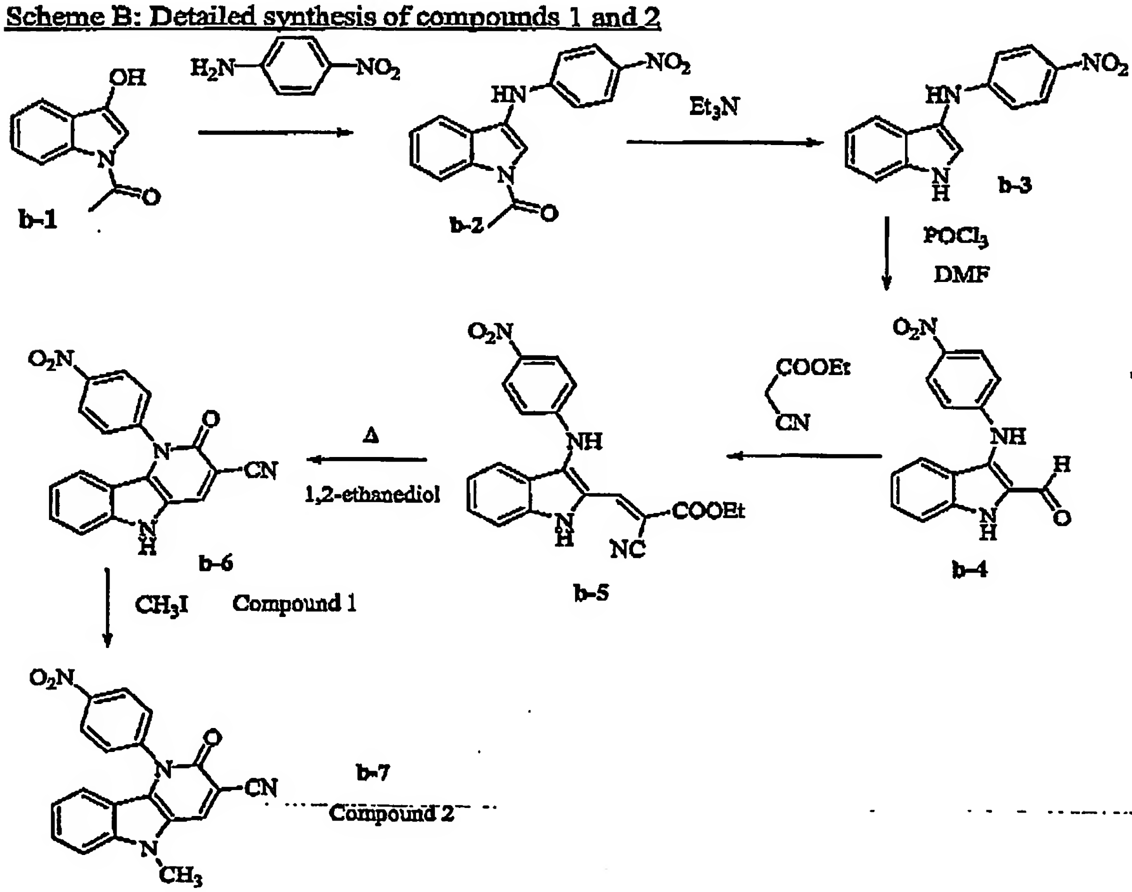
Figure 1: Time of addition experiment.
Y-axis: normalized virus production in %. X-axis: time of addition, in hours, of the compounds under investigation, following infection of the cells with HIV-LAI.

Figure 2: *In vitro* inhibition of reverse transcriptase.
Y-axis: percentage inhibition of HIV reverse transcriptase compared to control. X-axis: amount of compound added to wells in micromolar.

Experimental Part

Preparation of the compounds of formula (I) and their intermediates

Scheme B: Detailed synthesis of compounds 1 and 2



Synthesis of indolopyridinone compounds b-6 and b-7

The synthesis of compounds (b-6) and (b-7) started from the commercially available 1-acetyl-3-hydroxyindole (b-1). Condensation of intermediate (b-1) with 4-nitroaniline, under refluxing conditions in acetic acid, yielded 3-((4-nitrophenyl)amino)indole (b-2) (Valezheva et al.; Chem.Heterocycl.Compd.(Engl.Transl.); 14; 1978; 757,759,760; Khim.Geterotsikl.Soedin.; 14; 1978; 939). Deacylation of intermediate (b-2) with triethylamine in refluxing methanol and formylation of intermediate (b-3) using phosphorus oxychloride in dimetylformamide resulted in intermediate (b-4) (Ryabova, S. Yu.; Tugusheva, N. Z.; Alekseeva, L. M.; Granik, V. G.; Pharm. Chem. J. (Engl. Transl.); EN; 30; 7; 1996; 472 - 477; Khim.Farm.Zh.; RU; 30; 7; 1996; 42 – 46). Knoevenagel condensation of intermediate (b-4) with ethyl cyanoacetate in the presence of a catalytic amount of triethylamine and subsequent intramolecular cyclisation of intermediate (b-5) under reflux in 1,2-ethanediol, yielded compound (b-6) (1-(4-nitro-phenyl)-2-oxo-2,5-dihydro-1*H*-pyrido[3,2-*b*]indole-3-carbonitrile) (compound 1) (Ryabova, S. Yu.; Alekseeva, L. M.; Granik, B. G.; Chem. Heterocycl. Compd. (Engl.Translat.)36; 3; 2000; 301 - 306; Khim.Geterotsikl.Soedin.; RU; 3; 2000; 362 – 367). N-methylation using methyl iodide led to compound (b-7) (5-methyl-1-(4-nitro-phenyl)-2-oxo-2,5-dihydro-1*H*-pyrido[3,2-*b*]indole-3-carbonitrile) (compound 2).

Time of addition experiment

A time of addition experiment was performed to determine the mechanism of action of the compounds of the present invention. In the time of addition experiment, compounds are added to cells that were infected with HIV, at time zero (Zero hours). The compounds are subsequently added at different points in time. The time point until which a compound can be added to prevent virus replication, provides an indication of the mechanism of action of the compound.

In the present experiment, MT4 cells were infected with HIV-1 strain LAI at time zero. In different experiments, compounds were subsequently added at the points in time indicated in the X-axis of Figure 1 (in hours). The compounds were added at the following end concentrations during incubation: DS5000, 1 μM; efavirenz (EFV), 1 μM; saquinavir (SQV), 1 μM; Reference 1, 10 μM (Reference 1 is an integrase inhibitor selected from WO 99/62520 and is present in CAS database: 251963-93-6); Compound 2, 50 μM; Control: normalized virus production. The virus production was determined using p24 monitoring using a kit according to the manufacturers instructions (p24 ELISA kit, catalog reference NEK-050, Perkin Elmer).

Compound 2 delayed virus production using a mechanism related to reverse transcriptase.

<u>In vitro inhibition of HIV reverse transcriptase</u>

The assay was run using kit TRK 1022 (Amersham Life Sciences) according to the manufacturers instructions with slight modifications. Compounds were diluted in steps of 1/4 in 100% DMSO and subsequently transferred to Medium A (1/50 dilution; medium A: RPMI 1640 + 10% FetalClone II + Gentamycin 20 mg/L). 25 µl of compound (in 2% DMSO in Medium A) or 25 µl of 2% DMSO in medium A was added to wells. To each well was added 25.5 µl master mix (master mix: 5 µl primer/template beads, 10 µl assay buffer, 0.5 µl tracer (3H-TTP), 5 µl HIV RT enzyme solution at a final enzyme activity of 15 mU per 50 µl reaction, 5 µl medium A). The plates were sealed, marked as radioactive and incubated during 4 hours at 37°C. Subsequently, 100 µl stop solution was added to each well (except R1). The radioactivity was counted in a TopCount.

Compound 2 inhibits HIV reverse transcriptase *in vitro* and consequently does not need conversion to an active metabolite in order to inhibit reverse transcriptase.

<u>Metabolization of the compounds of the present invention</u>

The present experiment provides insight into the hepatic first pass metabolization of compounds.

Aliquots of human liver microsomal fractions (prepared by centrifugation at 12000 g^{-1}) were transferred into 10 ml glass tubes that are immersed in ice. Subsequently, test compound was added to yield a final concentration of 10 µM test compound. After adding 500 µl of a co-factor solution (cofactor solution: 1 mg/ml glucose-6-phosphate, 1 mg/ml $MgCl_2.6H_2O$, 0.5 units/ml glucose-6-phosphate dehydrogenase in 0.5 M phohsphate buffer pH 7.4), homogenisation buffer (homogenisation buffer: 1,15 % KCl in 0.05 M phosphate buffer, pH 7.4) was added to give a final volume of 1 ml. The incubations, 30 or 120 minutes at 37°C, were initiated by adding 10 µl of a solution of nicontinamide adenine dinucleotide phosphate (1,25 mg/ml) in homogenisation buffer. After a preincubation during 5 minutes at 37 °C, the tubes were continuously shaken at 100 oscillations /minute in a water bath. The reactions were terminated by addition of an equal volume of DMSO. Blank incubations containing boiled microsomal fractions were incubated under the same conditions as the drug incubations. The degree of metabolism was determined by direct measurement of the residual parent compound in the reaction mixture using LC-MS. In parallel, the residual anti-HIV activity in the

reaction mixture was detected using a colorimetric anti-HIV assay as described in Pauwels *et al.* J. Virol. Methods 1988 (20) 309-321. The residual activity is defined as the percent difference in EC_{50} between the drug incubations and the blank incubations.

The results in Table 2 indicate that compound 2 underwent little or no hepatic first pass metabolization.

Table 2. Microsomal metabolization of compound 2.
The amount of compound was determined using LC-MS at the time points indicated between brackets. The results are indicated as a % vis-à-vis the amount determined at the start of the experiment (time = 0).

Compound name	Compound 2
Concentration	10 µM
DLM (0 min) (in %)	100
DLM (30 min) (in %)	91
DLM (120 min) (in %)	108
HLM (0 min) (in %)	100
HLM (30 min) (in %)	98
HLM (120 min) (in %)	128

DLM: dog liver microsomes, HLM: human liver microsomes, min: minutes

Antiviral analyses:
The compounds of the present invention were examined for anti-viral activity in a cellular assay. The assay demonstrated that these compounds exhibited potent anti-HIV activity against a wild type laboratory HIV strain (HIV-1 strain LAI). The cellular assay was performed according to the following procedure.

HIV- or mock-infected MT4 cells were incubated for five days in the presence of various concentrations of the inhibitor. At the end of the incubation period, the replicating virus in the control cultures has killed all HIV-infected cells in the absence of any inhibitor. Cell viability was determined by measuring the concentration of MTT, a yellow, water soluble tetrazolium dye that is converted to a purple, water insoluble formazan in the mitochondria of living cells only. Upon solubilization of the resulting formazan crystals with isopropanol, the absorbance of the solution was monitored at 540 nm. The values correlate directly to the number of living cells

remaining in the culture at the completion of the five day incubation. The inhibitory activity of the compound was monitored on the virus-infected cells and was expressed as EC_{50} and EC_{90}. These values represent the amount of the compound required to protect 50% and 90%, respectively, of the cells from the cytopathogenic effect of the virus. The toxicity of the compound was measured on the mock-infected cells and was expressed as CC_{50}, which represents the concentration of compound required to inhibit the growth of the cells by 50%. The selectivity index (SI) (ratio CC_{50}/EC_{50}) is an indication of the selectivity of the anti-HIV activity of the inhibitor. Wherever results are reported as e.g. pEC_{50} or pCC_{50} values, the result is expressed as the negative logarithm of the result expressed as EC_{50} or CC_{50} respectively.

Because of the increasing emergence of drug resistant HIV strains, the present compounds were also tested for their potency against clinically isolated HIV strains harboring several mutations (Table 1 and 3). These mutations are associated with resistance to reverse transcriptase inhibitors and result in viruses that show various degrees of phenotypic cross-resistance to the currently commercially available drugs such as for instance AZT, didanosine, nevirapine, lamivudine and zalcibatine.

Results:

As a measure of the broad spectrum activity of the present compounds, the EC_{50} was determined. Table 3 shows the results of the antiviral testing of the respective compounds expressed in pEC_{50}. The fold resistance rounded to the nearest integer is mentioned between brackets.

As can be seen in this table, the present compounds are effective in inhibiting a broad range of mutant strains: Row A: pEC_{50} value towards mutant A, Row B: pEC_{50} towards mutant B , Row C: pEC_{50} towards mutant C, Row D: pEC_{50} towards mutant D, Row E: pEC_{50} towards mutant E, Row F: pEC_{50} towards mutant F, Row G: pEC_{50} towards mutant G, Row H: pEC_{50} towards mutant G, Row H: pEC_{50} towards mutant H, Row I: pEC_{50} towards mutant I, Row J: pEC_{50} towards mutant J, Row K: pEC_{50} towards mutant K, Row HIV-2: pEC_{50} towards mutant HIV-2, Row SIV (simian immunodeficiency virus): pEC_{50} towards mutant SIV. Row WT: pEC50 against wild type HIV-LAI strain. The toxicity (Tox) is expressed as the pCC_{50} value as determined with mock transfected cells. ND means not determined.

Table 3. Results of the toxicity testing and the resistance testing.

Strain	Compound 1	Compound 2

WT	6.5	7.6
A	5.6 (8)	7.0 (4)
B	5.9 (4)	7.5 (1)
C	5.6 (8)	7.1 (3)
D	6.0 (3)	7.3 (2)
E	5.7 (6)	7.2 (3)
F	5.9 (4)	7.4 (2)
G	6.2 (2)	7.2 (3)
H	5.8 (5)	6.9 (5)
I	6.1 (3)	7.2 (3)
J	5.8 (5)	6.9 (5)
K	6.5 (1)	7.0 (4)
HIV-2	5.2	6.6
SIV	5.1	6.5
Tox	<4.49	<4.49

For comparative purposes, 2-(dimethylamino)-4,5-dihydro-5-methyl-1-(4-nitrophenyl)-4-(2-oxopropyl)-1H-pyrido[3,2-b]indole-3-carbonitrile as mentioned in WO 02/055520 has a pEC_{50} for wild type HIV virus of 5.5 indicating an increase in potency for the compounds of the present invention ranging between about 1 and 2 log units.

<u>Oral availability in the rat and the dog</u>

The compounds were formulated as a 20 mg/ml solution or suspension in DMSO, PEG400 or cyclodextin 40% (CD40%) in water. For most experiments in the rat, three dosing groups were formed: 1/ single intraperitoneal dose at 20 mg/kg using the DMSO formulation; 2/ single oral dose at 20 mg/kg using the PEG400 formulation and 3/ single oral dose at 20 mg/kg using the cyclodextrin formulation. Blood was sampled at regular time intervals after dosing and drug concentrations in the serum were determined using a LC-MS bioanalytical method.

<u>Formulation</u>

Active ingredient, *in casu* a compound of formula (I), can be dissolved in organic solvent such as ethanol, methanol or methylene chloride, preferably, a mixture of ethanol and methylene chloride. Polymers such as polyvinylpyrrolidone copolymer with vinyl acetate (PVP-VA) or hydroxypropylmethylcellulose (HPMC), typically 5 mPa.s, can be dissolved in organic solvents such as ethanol, methanol methylene chloride. Suitably the polymer can be dissolved in ethanol. The polymer and compound solutions can be mixed and subsequently spray dried. The ratio of compound/polymer

can be selected from 1/1 to 1/6. Intermediate ranges can be 1/1.5 and 1/3. A suitable ratio can be 1/6. The spraydried powder, a solid dispersion, can subsequently be filled in capsules for administration. The drug load in one capsule can range between 50 and 100 mg depending on the capule size used.

Film-coated Tablets

Preparation of Tablet Core

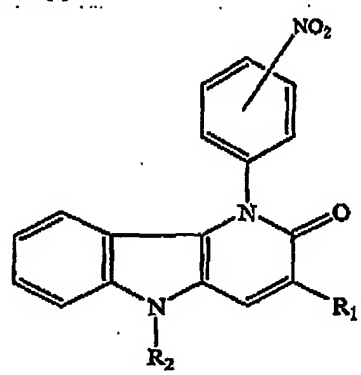
A mixture of 100 g of active ingredient, ***in casu*** a compound of formula (I), 570 g lactose and 200 g starch can be mixed well and thereafter humidified with a solution of 5 g sodium dodecyl sulfate and 10 g polyvinylpyrrolidone in about 200 ml of water. The wet powder mixture can be sieved, dried and sieved again. Then there can be added 100 g microcrystalline cellulose and 15 g hydrogenated vegetable oil. The whole can be mixed well and compressed into tablets, giving 10.000 tablets, each comprising 10 mg of the active ingredient.

Coating

To a solution of 10 g methylcellulose in 75 ml of denaturated ethanol there can be added a solution of 5 g of ethylcellulose in 150 ml of dichloromethane. Then there can be added 75 ml of dichloromethane and 2.5 ml 1,2,3-propanetriol. 10 g of polyethylene glycol can be molten and dissolved in 75 ml of dichloromethane. The latter solution can be added to the former and then there can be added 2.5 g of magnesium octadecanoate, 5 g of polyvinylpyrrolidone and 30 ml of concentrated color suspension and the whole can be homogenated. The tablet cores can be coated with the thus obtained mixture in a coating apparatus.

CLAIMS

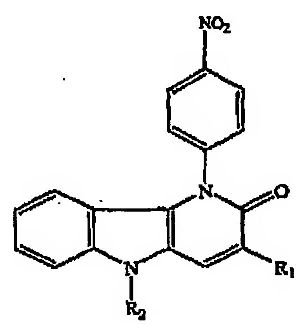
1. A compound of formula (I)



(I)

its *N*-oxide, salt, stereoisomeric form, racemic mixture, prodrug, ester or metabolite, wherein
R_1 is cyano, C_{1-4}alkyloxycarbonyl or C_{1-4}alkylaminocarbonyl;
R_2 is hydrogen or C_{1-6}alkyl;
for use as a medicine.

2. A compound of formula (I) as defined in claim 1 for use as a medicine, characterized in that it has general formula (II)



(II)

3. A compound of formula (I) as defined in claim 1 or 2 for use as a medicine, characterized in that R_1 is cyano, methyloxycarbonyl, methylaminocarbonyl, ethylaminocarbonyl, ethyloxycarbonyl.

4. A compound of formula (I) as defined in any of claims 1 to 3 for use as a medicine, characterized in that R_2 is hydrogen or methyl.

5. A compound of formula (I) as defined in any one of claims 1 to 4 for use as a medicine, characterized in that R_1 is cyano and R_2 is hydrogen or methyl.

6. Use of a compound of formula (I) as defined in any one of claims 1 to 5 for the manufacture of a medicament for preventing, treating or combating infection or disease associated with infection with HIV virus.

7. Use of a compound of formula (I) as defined in any one of claims 1 to 5 for the manufacture of a medicament for inhibiting the replication of HIV virus.

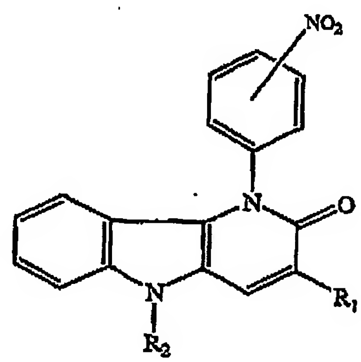
8. Use of a compound of formula (I) according to claim 6 or 7 characterized in that the reverse transcriptase of the HIV virus is mutant.

9. A pharmaceutical composition, comprising an effective amount of at least one compound of formula (I) as claimed in any one of claims 1 to 5, and a pharmaceutically tolerable excipient, for use as a medicament.

10. A process for the preparation of a composition as defined in claim 9.

11. A product containing at least one compound of formula (I) according to any one of claims 1 to 5 and an antiretroviral agent as a combined preparation for the simultaneous, separate or sequential use in antiretroviral therapy.

12. A compound of formula (I)



(I)

its *N*-oxide, salt, stereoisomeric form, racemic mixture, prodrug, ester or metabolite, wherein

R_1 is cyano, C_{1-4}alkyloxycarbonyl or C_{1-4}alkylaminocarbonyl;

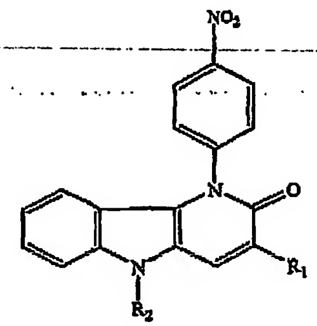
R_2 is hydrogen or C_{1-6}alkyl;

provided that the compound is different from

2,5-dihydro-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile, and

2,5-dihydro-5-methyl-1-(4-nitrophenyl)-2-oxo-1H-pyrido[3,2-b]indole-3-carbonitrile.

13. A compound according to claim 12, characterized in that it has general formula (II)



(II)

14. A compound according to claim 12 or 13 characterized in that when R_1 is cyano then R_2 is different from hydrogen or methyl.

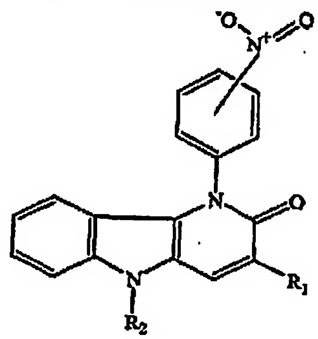
15. A compound according to any one of claims 12 to 14 characterized in that R_1 is C_{1-4}alkyloxycarbonyl or C_{1-4}alkylaminocarbonyl.

16. A compound according to any one of claims 12 to 15 characterized in that R_2 is C_{2-6}alkyl.

ABSTRACT

SUBSTITUTED INDOLEPYRIDINIUM AS ANTI-INFECTIVE COMPOUNDS

The present invention concerns the compounds of formula (I)



(I)

an *N*-oxide, salt, stereoisomeric form, racemic mixture, prodrug, ester or metabolite thereof, wherein, R_1 is cyano, C_{1-4}alkyloxycarbonyl or C_{1-4}alkylaminocarbonyl, and R_2 is hydrogen or C_{1-6}alkyl, for use as a medicine. The invention further relates to a novel subgroup of the compounds of formula (I), and to compositions comprising compounds of formula (I).

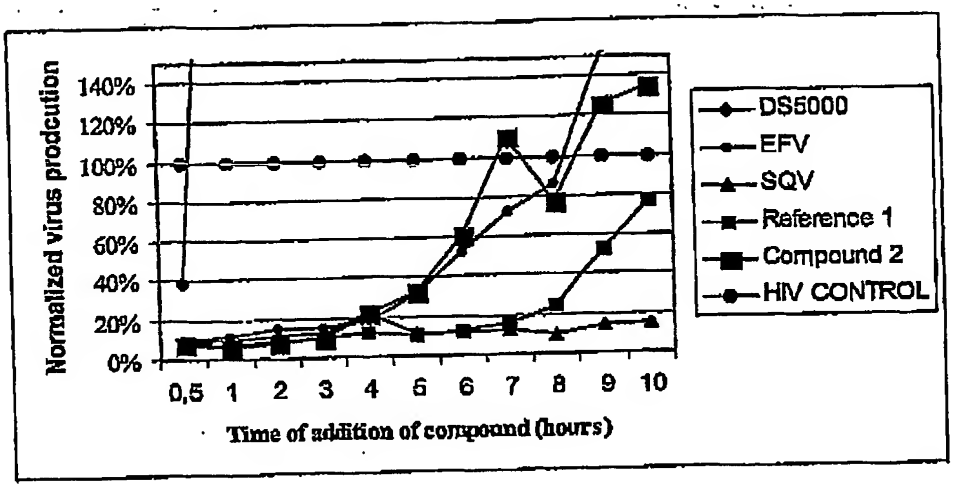


Figure 1

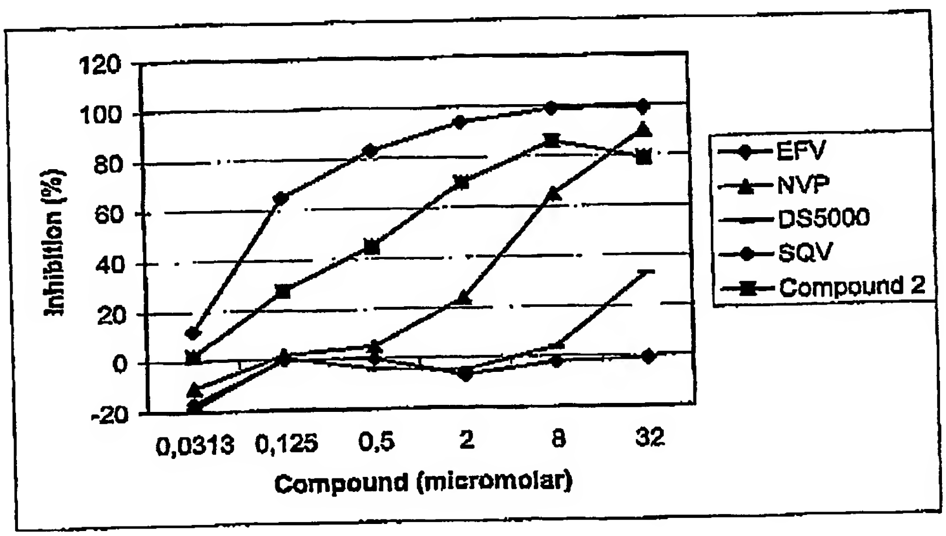


Figure 2